

## Physical, chemical and functional properties of gelatin extracted from the skin of rohu, *Labeo rohita* and yellowfin tuna, *Thunnus albacares*

GEORGE NINAN, A. A. ZYNUDHEEN, C. G. JOSHY AND K. SUMNA YOUSUF

Fish Processing Division, Central Institute of Fisheries Technology, Kochi - 682 029, Kerala, India

e-mail: george66jiji@gmail.com

### ABSTRACT

Gelatin, a commercially important polypeptide derived from collagen has wide applications in food and pharmaceutical industry. Gelatin extracted from the tropical fish species has an advantage over cold water species, the former having better rheological properties. In this study the physico-chemical properties of gelatin extracted from the skin of rohu, *Labeo rohita* and yellowfin tuna *Thunnus albacares* were studied. The results indicated that tuna skin gelatin was superior in terms of yield, gel strength, viscosity and foam stability. However, rohu skin gelatin had a better colour and foam formation ability. The amino acid composition showed significantly higher content of glycine (27.5%) and imino acid (26.98%) in tuna skin gelatin. Although tuna gelatin had better gel strength, rohu skin gelatin was found to be more suitable for food applications as it had better colour and sensory properties.

Keywords: Amino acids, Gelatin, Gel strength, Rohu, Skin, Yellowfin tuna

### Introduction

Search for new gelling agents to replace mammalian gelatin led to patents for fish gelatin production (Grossman and Bergman, 1992; Holzer, 1996) as well as several established methods for fish gelatin production (Gudmundsson and Hafsteinsson, 1997; Nagai and Suzuki, 2000; Gomez-Guillen and Montero, 2001; Arnesen and Gildberg, 2002). The commercial interest in fish gelatin has thus far however been relatively low due to sub-optimal physical properties compared to mammalian gelatin. However, recent studies have indicated that addition of co-enhancers significantly improve the gel strength and melting point of fish gelatin (Fernandez-Díaz *et al.*, 2001; Koli *et al.*, 2011). Warm water fish gelatins have properties quite similar to mammalian samples. Gelatin from the skin of yellow fin tuna, *Thunnus albacares* had a high gel strength (426 Bloom) in comparison with bovine and porcine gelatins while gelling and melting points were lower (Cho *et al.*, 2005). Jamilah and Harvinder (2002) reported bloom strength of 180.8 for gelatin extracted from black tilapia skin. The gelatin from channel catfish skin showed high gel strength of 276 Bloom (Liu *et al.*, 2008). Similarly gelatin from the skin of grass carp showed high contents of imino acids (19.47%) and medium gel strength 267 Bloom (Kasankala *et al.*, 2007). Type A gelatins extracted from skin and bones of young and adult Nile perch had Bloom values of 81–229 and 134–179 g, respectively (Muyonga *et al.*, 2004). Carp skin gelatin based films had significantly lower water vapour permeability and oxygen

permeability compared to mammalian gelatin films, which indicated the superior barrier properties of the latter (George *et al.*, 2010). These reports indicate that gelatin from fish resources of tropical waters have properties comparable with that of gelatin from mammalian origin. Production and utilisation of fish gelatin not only satisfies the needs of consumers, but also serves as a means to utilise some of the byproducts of the fishing industry (Karim and Bhat, 2009). The fishery waste generated from the processing of cultured Indian major carps can be a potential source for the production of gelatin. Rohu (*L. rohita*) and yellowfin tuna (*T. albacares*) represent two major commercially important species in the freshwater and marine environments respectively. Value addition of these species generates waste in the form of head, gills, entrails, skin and bone. Skin from these species is a good source of gelatin which can be exploited as a byproduct. The objective of the present study was to compare the physical, chemical and functional properties of gelatin extracted from the skin of rohu and yellow fin tuna.

### Materials and methods

#### Raw materials

Yellowfin tuna (*T. albacares*) skin was collected from the local processors in Cochin. The samples were brought to the laboratory in iced condition. The dorsal skin portion was used for the extraction. The skin of rohu (*L. rohita*) was collected as filleting discard from the pilot plant of Central Institute of Fisheries Technology, Cochin. All the chemicals used in this study were of analytical grade.

### *Extraction of gelatin from yellowfin tuna dorsal skin*

The extraction of gelatin was carried out following the procedure of Cho *et al.* (2005) with modifications. The tuna skin was washed and cut into fine pieces of 5-10 mm size. It was weighed and treated with 2.5% NaOH solution (1:8 ratio) at 22 °C with occasional stirring for two days to remove the non-collagen protein and subcutaneous tissues. After 48 h alkali was drained off, the skin neutralised with 6N HCl and washed. The gelatin was extracted with six volumes of distilled water at 60 °C for 10 h. The extracted solution was centrifuged (900 g) for 30 min at 30 °C. The upper phase was removed and freeze dried in a Freeze Drier (Gamma 1-16 LSC, Martin Christ, Germany) to obtain dry gelatin having a moisture content of <3%.

### *Extraction of gelatin from rohu skin*

The gelatin extraction procedure followed was essentially as described by Gudmundsson and Hafsteinsson (1997) with slight modifications. Cleaned skins were soaked in 0.2% (w/v) sodium hydroxide solution for 45 min, followed by soaking in 0.2 % (w/v) sulphuric acid for 45 min. After each treatment, the skins were washed under running water to near neutral pH. Each soaking and washing treatment was repeated two times. The ratio of skin to alkali/acid solution was 250 g wet weight of the skin to 1.5 l. of solution. The skins were then subjected to a final wash with distilled water before the final extraction. The final extraction was carried out in distilled water at controlled temperature of 45 °C. using a water bath (Julabo TW 20, Germany) for 10 h. The ratio used was 250 g wet weight of the skin to 1.5 l of distilled water. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (No. 4). The gelatin sample was finally prepared by freeze drying in a Freeze Drier (Martin Christ, Gamma 1-16 LSC, Germany).

### *Determination of yield*

The yield was calculated as described by Muyonga *et al.* (2004). For this, 10 ml of gelatine in duplicate was centrifuged, filtered and evaporated for determining solid concentration. The following equation was used for gelatine yield calculation:

$$\text{Yield} = \frac{C * V}{100} * M$$

where C = light liquor concentration (g ml<sup>-1</sup>), V = liquor volume, M = weight of skin sample (g) used for extraction.

### *Proximate composition and pH*

The moisture, protein, fat and ash contents of the extracted gelatins were determined following AOAC

(1995). For protein determination, a nitrogen conversion factor of 5.4 was used as per Eastoe and Eastoe (1952). The pH of gelatin solution was measured using the British Standard Institution method, BSI 757 (1975).

### *Amino acid composition, gel strength, colour and viscosity*

Total amino acids in gelatin samples were determined as per the procedure of Ishida *et al.* (1981) using Shimadzu Amino acid analyser (HPLC- LC 10 AS) equipped with cation exchange column packed with a strongly acidic cation exchange resin. The gel strength (Bloom) was determined by the British Standard 757: 1975 method (BSI, 1975) using a texture analyser (Lloyd Instruments, Model LRX Plus, U.K.) Colour analysis was performed with a Hunter lab Miniscan ® XE plus spectrophotometer (Hunter Associates Laboratory, Inc. Reston, Virginia, USA). Measurements were recorded using the L\* a\* b\* colour scale (CIE, 1986). Viscosity was measured as per the method described by Cho *et al.* (2005). The viscosity (cP) of 10 ml of the gelatin solution of 6.67% (w/v) was determined using Brookfield digital viscometer (Model DV E Brookfield Engineering, USA) equipped with a No.1 spindle at 30 ± 0.5 °C.

### *Sensory evaluation of gelatin samples*

Determination of odour by sensory evaluation was conducted as per the method of Muyonga *et al.* (2004) using a ten member panel. Samples were prepared by dissolving 0.5 g of gelatin in 7 ml of distilled water to obtain a solution containing approximately 6.67% gelatin. The samples were prepared in test tubes with screw caps and dissolved as described for the Bloom samples. The samples were held in a water bath at 50 °C, with the screw caps lightly closed. Panelists were instructed to remove the screw caps, sniff the contents and identify the odour they perceived as well as indicate the odour intensity, using a six point scale (0 = no odour, 1 = very mild and only perceivable on careful assessment, 2 = mild but easily perceivable, 3 = strong but not offensive, 4 = strong and offensive, 5 = very strong and very offensive).

### *Foam formation capacity, foam stability, water-holding and fat-binding capacities, melting point, gel setting point and setting time*

Foam formation capacity, foam stability, water-holding as well as fat-binding capacities were determined. based on the methods described by Cho *et al.* (2004). Melting point was measured as per Wainwright (1977). The method used for the determination of gel setting point and setting time of gelatin was that described by Muyonga *et al.* (2004). Gelatin solutions of 10% (w/w) were prepared in thin wall (12 mm × 75 mm) test tubes in the same way as described for the Bloom samples. The dissolved samples from the

warm water bath were transferred to another water bath held at 40 °C (circulating bath – Haake D3, Germany). The bath was then cooled slowly at the rate of 0.2 °C per min. A thermometer was inserted into the sample and lifted out at 30 seconds intervals. The temperature of the mixture at which the gelatin solution no longer dripped from the tip of the thermometer was recorded as the setting temperature. Setting time was determined on samples prepared in the same way as those for the determination of the setting temperature. Samples were transferred to a water bath maintained at 10 °C (circulating bath – Haake D3 Germany). A rod was inserted in the gelatin solution and raised at intervals of 15 seconds. The time at which the rod could not detach from the gelatin sample was recorded as the setting time.

#### Statistical analysis

All data were analysed using the analysis of variance (ANOVA) and Duncan's multiple tests to determine the significant difference between the means. Statistical package used in the study was SAS, Version 6 (1989). All the data represented are the means of triplicates.

## Results and discussion

#### Proximate compositions and pH

Crude protein content was in the range of 92-93% for the gelatins (Table 1) which is higher than that reported for other fish gelatins (Muyonga *et al.*, 2004; Jongjareonrak *et al.*, 2006). Protein content in the range of 81-96% was reported for tuna gelatin by many workers (Aewsiri *et al.*, 2008; Pranoto *et al.*, 2011). Moisture content in all the samples was below 3% since the gelatins were subjected to freeze drying. The ash content in all the samples were in the range of 1.7 - 1.8%, less than the recommended maximum limit of 2% set for edible gelatin (GME, 2008). Fat content was significantly higher ( $p < 0.05$ ) for rohu gelatin. The pH values were 5.08 and 5.22 respectively for rohu and yellowfin tuna gelatins and hence these can be categorised as Type B gelatins.

Table 1. Proximate composition and pH of extracted gelatin samples\*

Parameters	Rohu gelatin	Yellowfin tuna gelatin
Moisture (%)	2.51 (0.15)	2.63 (0.13)
Protein (%)	92.43 (0.70)	93.65 (0.88)
Lipid % (DWB)	2.57 (0.07) <sup>a</sup>	1.21 (0.17) <sup>b</sup>
Ash (%)	1.70 (0.14)	1.81 (0.11)
pH	5.08 (0.04) <sup>b</sup>	5.22 (0.02) <sup>a</sup>

\*Values in parentheses are standard deviations of triplicate analysis

<sup>a-b</sup> Means within a row with different letters are significantly different ( $p < 0.05$ )

#### Physical properties

The yield of gelatin from yellow fin tuna skin was significantly higher ( $p < 0.05$ ) than that from rohu skin (Table 2). Yellowfin tuna gelatin had significantly higher values ( $p < 0.05$ ) for viscosity, melting temperature, setting temperature and setting time than rohu gelatin. The viscosity of the gelatins samples were close to that prescribed for commercial gelatin *i.e.*, around 7 cP. Yellowfin tuna gelatin had a melting point of 29.27 °C which was higher than the values reported by others (Cho *et al.*, 2005; Pranto *et al.*, 2011). The gel setting temperature was 18.8 °C and the gel setting time was significantly faster for yellowfin tuna. In this study yellowfin tuna showed significantly higher gelling and melting points than rohu gelatin and also most of the warm water fish sources reported in earlier studies (Gilsenan and Ross-Murphy, 2000; Gudmundsson, 2002; Jamilah and Harvinder, 2002; Pranto *et al.*, 2011). The gelling and melting temperature of gelatin has been found to correlate with the proportion of the imino acids proline and hydroxyproline in the original collagen (Piez and Gross, 1960; Veis, 1964; Ledward, 1986).

Table 2. Physical properties of extracted gelatin samples\*

Properties	Rohu gelatin	Yellowfin tuna gelatin
Gelatin yield (%)	12.93 (0.84) <sup>b</sup>	15.86 (1.2) <sup>a</sup>
Viscosity (cP)	6.06 (0.02) <sup>b</sup>	7.17 (0.05) <sup>a</sup>
Melting temperature (°C)	28.13 (0.03) <sup>b</sup>	29.27 (0.06) <sup>a</sup>
Gel Setting temperature (°C)	18.52 (0.10) <sup>b</sup>	18.80 (0.14) <sup>a</sup>
Setting time (seconds)	106.00 (3.7) <sup>a</sup>	90.00 (4.5) <sup>b</sup>
Sensory score	2.30 (0.12) <sup>b</sup>	3.11 (0.12) <sup>a</sup>

\*Values in brackets are standard deviations of triplicate samples.

<sup>a-b</sup> Means within a row with different superscripts are significantly different ( $p < 0.05$ )

Colour*	L*	a*	b*
Rohu gelatin	91.89 (0.62) <sup>a</sup>	-0.35 (0.01) <sup>b</sup>	1.76 (0.21) <sup>b</sup>
Yellowfin tuna gelatin	76.55 (0.82) <sup>b</sup>	2.20 (0.02) <sup>a</sup>	3.28 (0.35) <sup>a</sup>

\*Values in brackets are standard deviations of triplicate samples.

<sup>a-b</sup> Means within a column with different superscripts are significantly different ( $p < 0.05$ )

The gelatin prepared from the skins of rohu was found to be better in sensory score as it had a mild but easily perceivable odour. whereas yellowfin tuna gelatin had a strong odour (Table 2). Muyonga *et al.* (2004) reported that the gelatins prepared from the skin and bone of Nile perch were found to be free of fishy odour and to have a mild putrid odour with a mean hedonic score of 2–2.5 with activated carbon treatment. Strong fishy odour was reported for freeze-dried gelatin prepared from the skin of black tilapia (Jamilah and Harvinder, 2002).

The 'L\*', a\* and b\* values for rohu and yellowfin skin gelatin were 91.89, - 0.35 & 1.76 and 76.55, 2.20 & 3.28 respectively. The gelatin from the skin of rohu had a snowy white appearance and was light textured than yellowfin tuna gelatin. The colour of the gelatin depends on the raw material used for the extraction and also whether it is obtained from first stage, second stage or subsequent stages. Yellowfin tuna gelatin showed significantly lower value ( $p < 0.05$ ) for lightness ('L\*') than the rohu gelatin. The a\* values for rohu gelatin sample had negative values, indicating a shift of colour toward green. The b\* values were positive indicating the degree of yellowness. Rohu gelatin had significantly low b\* value than yellowfin tuna gelatin. This could be a positive attribute, since it is easier to incorporate these gelatins into any food system without imparting any strong colour attribute to the product. Similar colour values were reported for freeze dried gelatins from the skin of tilapia by Jamilah and Harvinder (2002).

The amino acid composition of the gelatins extracted from rohu and yellowfin tuna are given in Table 3. Rohu and yellowfin tuna skin gelatin had high content of imino acids, *i.e.*, 22.49 and 26.98% respectively. The imino acid content is approximately 30% for mammalian gelatins, 22–25% for warm water fish gelatins and 17% for cold-water fish gelatin (Muyonga *et al.*, 2004). High content of

Table 3. Amino acid composition of the extracted gelatin

Amino acids	Amino acids (g 100 g protein <sup>-1</sup> )	
	Rohu gelatin	Yellow fin tuna gelatin
Aspartic acid	2.56	2.18
Threonine	5.41	2.38
Serine	5.69	2.16
Glutamic acid	11.63	10.17
Proline	13.59	17.77
Glycine	25.93	27.51
Alanine	2.16	7.44
Cysteine	ND	ND
Valine	2.62	2.21
Methionine	2.43	1.52
Isoleucine	0.15	1.22
Leucine	3.21	2.56
Tyrosine	0.48	0.32
Phenylalanine	2.22	2.11
Histidine	0.40	0.62
Lysine	2.83	3.5
Arginine	4.93	6.67
Hydroxyproline	8.90	9.21
Imino acids (Pro + Hyp)	22.49	26.98
Total	95.14	99.55

imino acids improves the rheological properties of gelatin as it is involved in the formation of triple helical regions that immobilise water (Christopher, 1993). Glycine constitutes 25.93 and 27.51% of the total amino acid residues in rohu and yellowfin tuna skin gelatins respectively. Higher percentage of glycine leads to better water binding of the gelatin which is normally indicated by high viscosity, gel strength and melting point.

#### Functional properties

Gel strength is one of the most important functional properties of gelatin and fish gelatin typically has less gel strength than mammalian gelatin (Gilsenan and Ross-Murphy, 2000). Yellowfin tuna gelatin had significantly higher gel strength ( $p < 0.05$ ) compared with rohu gelatin (Table 4). The gel strength for yellowfin tuna skin gelatin is 315.98 Bloom which is higher than those reported by Pranoto *et al.* (2011) (159.03 and 163.36 Bloom from fresh and dried skins) and Gómez-Estaca *et al.* 2009 (167 Bloom). Higher imino acid content can be attributed to the better gel strength of yellowfin tuna skin gelatin than rohu gelatin.

Table 4. Functional properties of the extracted gelatin samples\*

Properties	Rohu gelatin	Yellow fin tuna gelatin
Gel strength (Bloom) (g)	188.63 (2.64) <sup>b</sup>	315.98 (2.64) <sup>a</sup>
Foam formation ability (FA)	2.55 (0.14) <sup>a</sup>	0.38 (0.03) <sup>b</sup>
Foam stability (FS)	1.83 (0.12) <sup>a</sup>	1.40 (0.11) <sup>b</sup>
Water holding capacity (ml g <sup>-1</sup> )	1.92 (0.30) <sup>a</sup>	1.77 (0.10) <sup>b</sup>
Fat binding capacity (ml g <sup>-1</sup> )	4.57 (0.55) <sup>b</sup>	4.96 (0.61) <sup>a</sup>

\*Values in parentheses are standard deviations of triplicate analysis. Values with different superscripts within a row are significantly different ( $p < 0.05$ )

Foam formation ability (FA) and foam stability (FS) of rohu and yellowfin tuna gelatins are given in Table 4. FA of yellow fin tuna gelatin was significantly ( $p < 0.05$ ) lower than rohu gelatin. However, tuna gelatin had better foam stability than rohu gelatin. The reduced foam formation ability may be due to the aggregation of proteins which interfere with the interactions between the protein and water (Kinsella, 1977). Rohu skin gelatin had higher WHC and lower FBC than yellow fin tuna gelatin. FBC of gelatin depends on the degree of exposure of the hydrophobic residues inside the gelatin.

Fish processing waste, particularly skin from commercially important species *viz.*, rohu and yellowfin tuna are good sources of gelatin with medium to high gel strength. Rohu skin gelatin was superior to yellowfin tuna skin gelatin in terms of sensory and colour attributes and can be potential replacer for mammalian gelatins in food applications. Yellowfin tuna skin gelatin can be used in the preparation of hard gel capsules.

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